Cyclophilin Content of Normal and Psoriatic Epidermis

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The unique, immunosuppressive agent cyclosporine A has been shown to be of important therapeutic value in the treatment of psoriasis and other inflammatory dermatoses. To investigate the basis for its therapeutic efficacy, the tissue and cellular content of cyclophilin, a cytosolic receptor for cyclosporine A, has been determined in keratome biopsies from normal and psoriatic epidermis and in cultured, adult human keratinocytes. The mean cyclophilin content of normal ($n = 10$), involved ($n = 10$), and uninvolved ($n = 10$) psoriatic epidermal samples was not significantly different (0.126 ± 0.016, 0.106 ± 0.009, and 0.153 ± 0.018 μg cyclosporine A bound/mg cytosolic protein). Similarly, the cyclophilin content of keratinocytes cultured from normal and psoriatic epidermis (0.21 ± 0.016 and 0.18 ± 0.024 μg/mg protein, respectively) did not differ significantly.

Western blot analysis of normal and psoriatic epidermal extracts with monospecific rabbit anti-cyclophilin antisera revealed a single band of 17,000 daltons, which co-migrated with highly purified bovine cyclophilin. The intensity of this band was similar in normal and psoriatic samples, indicating that immunoreactive cyclophilin content was similar. Cyclophilin mRNA was readily detected in normal and involved psoriatic epidermis by RNA blot hybridization, and expression was not significantly different in normal and psoriatic epidermis.

These findings indicate that cyclophilin is present in human keratinocytes and epidermis, where it may account for the accumulation of cyclosporine during therapy. However, the similar cyclophilin content of normal and diseased tissue suggests that differences in cyclosporine uptake probably do not account for its therapeutic efficacy in psoriasis. *J Invest Dermatol 94:436–440, 1990*

Cyclosporine A (CsA), a fungal undecapeptide and potent immunosuppressant [1] has proved to be a useful therapeutic agent in the treatment of psoriasis [2,3]. The mechanism of CsA immunosuppression has not been fully elucidated, but appears to reside, in part, in its ability to inhibit early events in interleukin-1-induced T-lymphocyte activation [4]; and by the inhibition of $CD4^+$ T-lymphocyte production of lymphokine mRNA, in particular that encoding for interleukin-2 [5] and gamma interferon [6].

Molecular targets for CsA have been identified that may account for both its accumulation by cells and the biochemical basis for its action. Cyclophilin (Mr 17,737, pI 9.4–9.6) has been isolated from bovine thymus and human spleen [7]. Cyclophilin is known to have a highly conserved structure at the levels of mRNA and protein [8] and nearly ubiquitous tissue and cellular distribution [9]. Recently it has been demonstrated that cyclophilin and peptidyl-prolyl cis-trans isomerase (PPIase), an enzyme important in catalyzing the refolding of several proteins, are probably identical [10,11]. Calmodulin, another ubiquitous protein, may also bind CsA (Kd 1×10^{-6} to 2×10^{-7} M) in fluid phase assays [12]; however, a solid phase assay has failed to demonstrate CsA binding [13]. Binding of CsA to the prolactin receptor has also been reported [14]. In spite of this evidence, the relationship of cellular and tissue levels of CsA and the functional consequence of CsA interaction with these target molecules has not been established.

The accumulation of CsA in psoriatic epidermis [15] and its therapeutic efficacy in psoriasis [2,3] suggest the participation of one or more CsA target molecules. We have focused on cyclophilin and examined tissue and cellular content in normal and psoriatic epidermis and cultured keratinocytes by a previously described Sephadex LH-20 column CsA-binding assay [9,16,17]. RNA blot hybridization and Western blot analysis were utilized to confirm the identity and relative abundance of cyclophilin mRNA and protein in normal and psoriatic epidermis.

MATERIALS AND METHODS

Materials All chemicals used, unless otherwise stated, were purchased from the Sigma Chemical Co., St. Louis, MO. [^{3}H]CsA was supplied by Sandoz Pharmaceuticals, Basle, Switzerland.

Tissue Samples Epidermal samples were obtained by keratome biopsy (2×5 cm) after 1% lidocaine anesthesia from normal ($n = 10$) and untreated psoriatic volunteers ($n = 10$). Biopsies were taken from either trunk, abdomen, buttocks, or thighs. The keratome biopsies were snap-frozen in liquid nitrogen and maintained at $-70°C$ until homogenized. The keratome depth for epidermal samples from involved psoriatic skin was 0.3–0.5 mm and 0.2 mm from uninvolved or normal skin. This study was approved by the University of Michigan Institutional Review Board and all subjects gave written, informed consent.

Preparation of Cytosolic Extract Epidermal samples were first washed in homogenization buffer (150 mM KCl, 20 mM Tris, 5 mM 2-mercaptoethanol, pH = 7.2) minced with scissors, ho-

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mogenized in a polytron and then a Potter glass homogenizer with a glass pestle at 4°C. The homogenate was centrifuged at 120,000 × g for 40 min at 4°C, and the supernatant used as the source for cytosolic cyclophilin. Protein content of the homogenates was determined by the Bio-Rad protein assay kit using bovine gamma globulin as standard [18].

Measurement of Cyclophilin Activity Cyclophilin activity was determined by a previously characterized [^{3}H]CsA binding assay [16,17] using a Sephadex LH-20 column to separate bound from free radio-ligand. Mini-columns (3 ml) of Sephadex LH-20 resin were pre-equilibrated with 10 ml assay buffer (20 mM Tris, 5 mM 2-mercaptoethanol, pH = 7.2). Samples of cytosol (82.5 μl) were added to 12 × 75 mm glass test tubes containing 7.5 μl of fetal bovine serum. [^{3}H]CsA (50 μg/ml) in 10 μl of 40% ethanol (15,000 cpm) was added (4 μM final concentration) and the tubes agitated briefly at room temperature. Control samples contained 82.5 μl homogenizing buffer, 7.5 μl fetal bovine serum, and 10 μl [^{3}H]CsA. Non-specific binding was determined by the addition of 100-fold excess unlabeled CsA. Aliquots of the incubation mixture (50 μl) were applied to the columns. Samples were washed into the column with 100 μl and then 350 μl of assay buffer. Columns were eluted with a further 2 ml of assay buffer and 5,500 μl fractions were collected. The cyclophilin-[^{3}H]CsA complex was eluted in the 1–1.5 ml fraction. The radioactivity of all five fractions was measured after addition of 5 ml of Safety-Solve scintillation fluid (Research Products International Corp., Mount Prospect, IL). The amount of [^{3}H]CsA binding activity was calculated as μg CsA bound/ml = cpm − background/[^{3}H]CsA specific activity × volume of cytosol assayed. All data are expressed as specific activity, μg CsA bound per mg protein.

A competitive displacement assay was performed according to the method described by Koletsky et al [9] except that 0.025 μg CsA binding activity was employed. Both normal and involved psoriatic epidermis were assayed. Binding of [^{3}H]CsA was assayed using the Sephadex LH-20 column. Binding of [^{3}H]CsA to normal epidermal cytosol was calculated as a function of [^{3}H]CsA concentration.

Determination of Cyclophilin Content of Keratinocytes Single-cell suspensions of normal keratinocytes were prepared from keratome biopsies of normal epidermis by trypsinising (0.3% trypsin and 0.1% EDTA) for 30 min at 37°C. Psoriatic keratinocytes were prepared from 6-mm punch biopsies taken from psoriatic plaques and trypsinized overnight at 20°C. Small, round viable cells were seeded onto, and grown in, 10 cm diameter plastic, petri dishes (Lux Flow Laboratories, Inc.) in a serum-free keratinocyte growth medium containing low calcium [19] (KGM, Clonetics, Co., San Diego, CA). Cells were maintained in a humidified incubator with 5% Co_2/95% air at 37°C. The keratinocytes from one 10-cm plate (3rd passage) were used per assay. Cells were scraped from the dish in 1 ml of homogenizing buffer and disrupted by sonication. The homogenate was then centrifuged at 120,000 × g for 40 min at 4°C and the cyclophilin content of the supernatant determined as described above.

Electrophoresis and Immunoblotting Extracts from epidermal biopsies (200 μg protein), homogenized in KCl-free homogenization buffer, were subjected to 12% sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to nitrocellulose membranes [20]. Nitrocellulose membranes were incubated for 2 h with anti-cyclophilin IgG fraction from ammonium sulphate precipitated rabbit serum (supplied by Dr. M.W. Harding) at a dilution of 1:1000. Following incubation with [^{125}I]protein A (3.1 μC/μl) for 2 h, the blots were developed at −70°C with an intensifying screen for 12 h. The pre-stained molecular weight standards (BRL Inc., Gaithersburg, MD) were insulin (M_r 3,100), bovine trypsin inhibitor (M_r 6,200), lysozyme (M_r 14,500), B-lactoglobulin (M_r 17,900), A-chymotrypsinogen (M_r 25,500), and ovalbumin (M_r 43,000). Purified bovine cyclophilin [7], M_r 17,737, was used as control.

RNA Blot Hybridization Analysis The procedures used for RNA extraction, quantitation, hybridization, autoradiography, and densitometric analysis have been described [21]. The probe was derived from pCD15:8-1 [8] by recloning at the Bam Hl site in a pGEMM 4Z vector.

Statistical Analysis The Scheffe one-way analysis of variance was used to compare the cyclophilin activity of normal and involved and uninvolved psoriatic epidermis and normal and psoriatic keratinocytes.

RESULTS

Cyclophilin Content of Epidermis and Keratinocytes from Normal and Psoriatic Patients A cyclosporine binding assay was utilized to measure the content of cyclophilin in soluble extracts from normal and psoriatic epidermis and keratinocytes [16,17]. Cyclophilin-[^{3}H]CsA complexes were specifically eluted from the LH-20 column (3 ml) in the 1.0–1.5 ml fraction, whereas unbound [^{3}H]CsA eluted between 2.5 and 5.0 ml (Fig 1). Using this technique the elution time is small compared with the dissociation rate constant. Addition of 100-fold excess unlabeled CsA reduced the radioactivity eluting in the bound fraction (1–1.5 ml) by greater than 95%. This indicates that the observed elution profile of [^{3}H]CsA in the presence of epidermal cytosol is due to specific binding. Binding of [^{3}H]CsA to normal and psoriatic epidermal cytosol was linear with respect to sample concentration between 50 and 200 μg protein (Fig 2). Binding of [^{3}H]CsA was also dose dependent with half-maximal and maximal binding at 0.13 and 0.6 μM CsA, respectively (Fig 3).

Data in Fig 4 show the cyclophilin content of normal and psoriatic epidermis. There was no significant difference in the mean cyclophilin content among normal (n = 10), psoriatic uninvolved

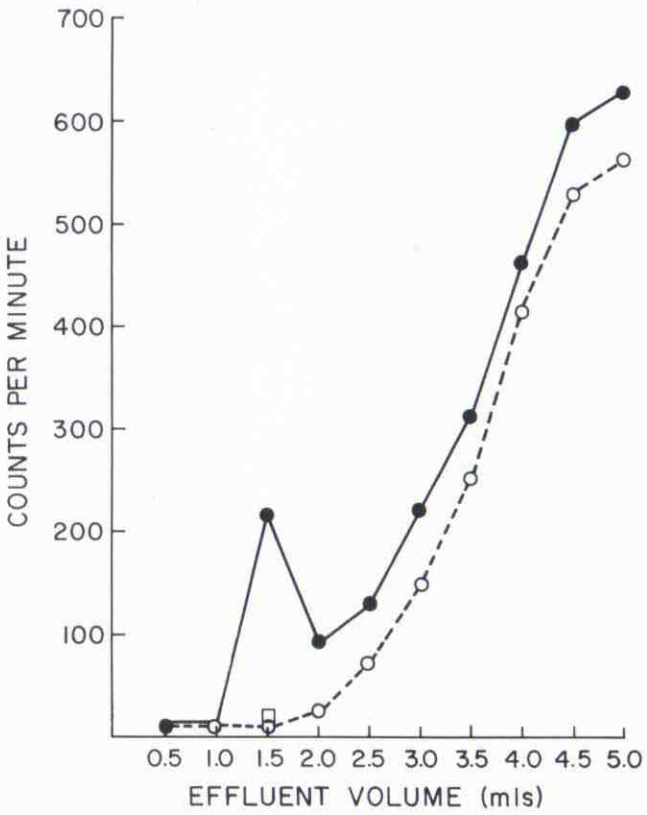

Figure 1. Binding of [^{3}H]CsA to cyclophilin from normal human epidermis. Cyclophilin-[^{3}H]CsA complexes were eluted from Sephadex LH-20 in the 1–1.5 ml fraction with non-complexed [^{3}H]CsA eluting after 2 ml. (*Closed circles:* normal cytosolic fraction; *open circles:* control buffer only; *open square:* binding in the presence of 20-fold excess of unlabeled CsA.)

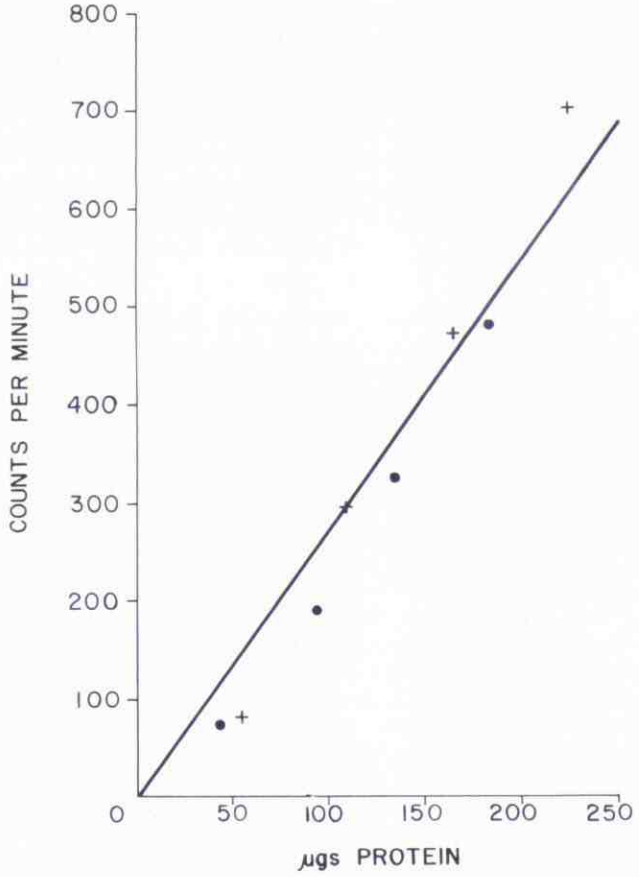

Figure 2. Binding of [^{3}H]CsA to normal and psoriatic involved epidermal high-speed supernatant as a function of protein concentration. [^{3}H]CsA binding was determined by LH-20 column assay. *Closed circle*, normal epidermis; *plus*, psoriatic plaque.

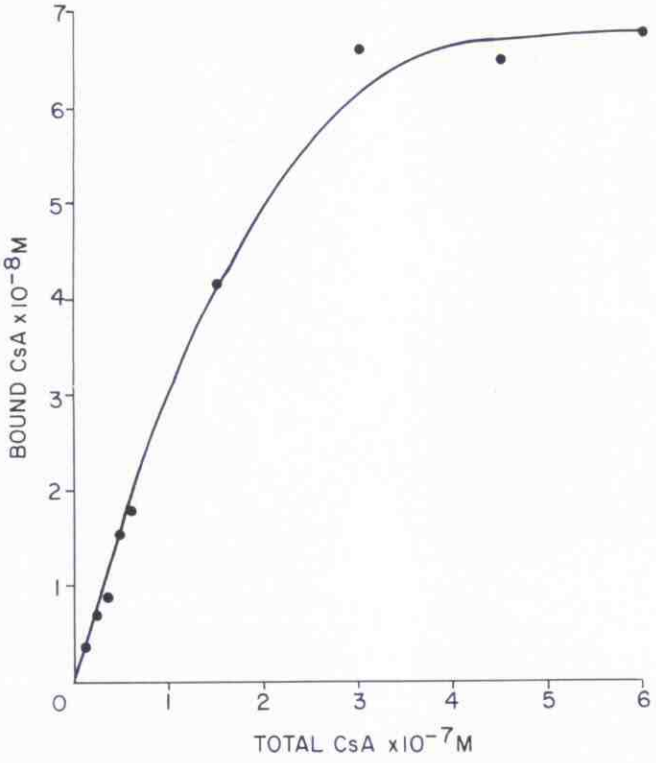

Figure 3. Binding of [^{3}H]CsA to normal epidermal high-speed supernatant as a function of [^{3}H]CsA concentration. Binding of [^{3}H]CsA was assayed by Sephadex LH-20 column.

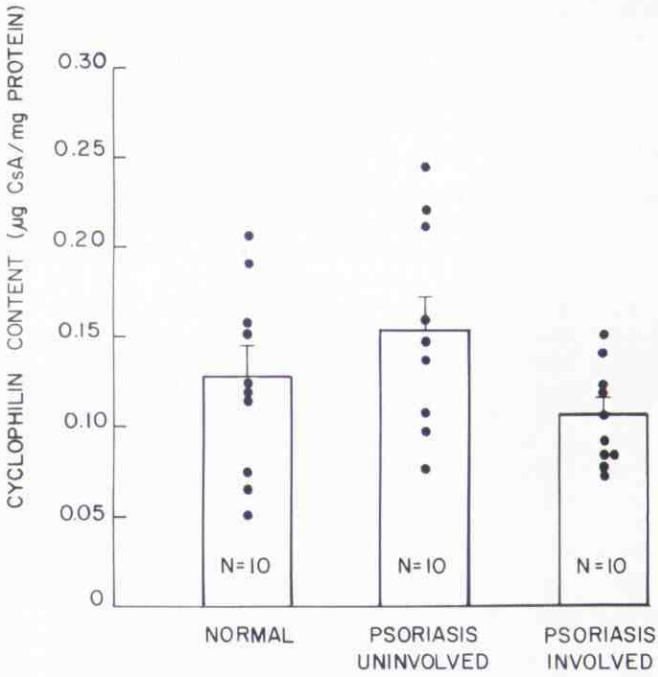

Figure 4. Cyclophilin content (µg CsA bound/mg protein) of normal psoriatic involved and psoriatic uninvolved epidermis as determined by Sephadex LH-20 column assay. There are no significant differences between the values for normal and psoriatic epidermis. (Values, mean ± SEM.)

(n = 10), and psoriatic involved (n = 10) epidermis, the mean values, in µg CsA bound/mg protein, being 0.126 ± 0.016, 0.153 ± 0.018, and 0.106 ± 0.009, respectively. Neither was there a significant difference in the cyclophilin content between normal and psoriatic cultured keratinocytes, 0.211 ± 0.06 µg CsA bound/mg and 0.182 ± 0.024 µg CsA bound/mg, respectively (Fig 5).

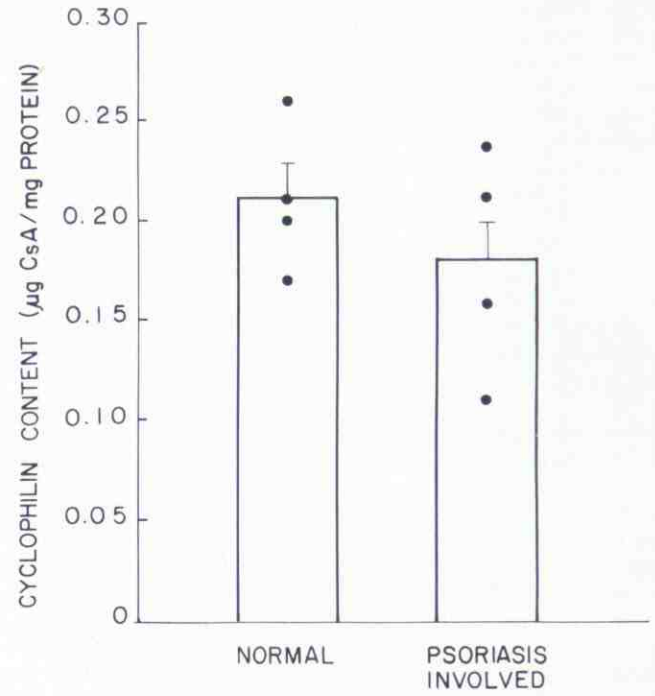

Figure 5. Cyclophilin content (µg CsA bound/mg protein) of normal and psoriatic cultured keratinocytes as determined by Sephadex LH-20 column assay. There is no significant difference between the values for normal and psoriatic keratinocytes. (Values, mean ± SEM.)

Identification of Immunoreactive Cyclophilin in Epidermal Extracts by Immunoblotting The identity of cyclophilin in epidermis and its relative content was further evaluated by immunoblotting. Cytosolic protein extracts from normal psoriatic involved and psoriatic uninvolved epidermal samples were resolved by SDS-PAGE and immunoblotted as described. Homogeneous bovine cyclophilin and cyclophilin in the epidermal cytosol extracts were identified by rabbit anti-cyclophilin immune serum. A protein band of M_r approximately 17,000 was identified in the epidermal extracts (data not shown). This band co-migrated with purified bovine cyclophilin, which has a calculated molecular weight (based on amino acid sequence) of 17,737. Equal protein loads of epidermal extract for the three sample groups displayed similar band intensity, indicating equivalent immunoreactive cyclophilin content.

Identification and Quantification of Cyclophilin mRNA Northern blots of total RNA derived from keratome biopsies of normal and lesional psoriatic epidermis were hybridized to the cyclophilin cDNA probe. The appropriate 1.0-Kb hybridizing bands are shown in Fig 6. One of the psoriatic samples (*fourth lane* from left) was noted to be underloaded by inspection of the ethidium bromide stained gel (data not shown). The mean integrated autoradiographic intensities of the hybridizing bands in normal and lesional psoriatic epidermis were not significantly different ($p > 0.05$ by two-tailed Student t test).

DISCUSSION

This study has demonstrated, for the first time, positive evidence for the presence of CsA binding, immunoreactive cyclophilin, and cyclophilin mRNA in normal and diseased human epidermis. The molecular weight and cytosolic content determined for epidermal cyclophilin are similar to that of other normal and neoplastic tissues [9]. There was no significant difference in the cyclophilin content of epidermal samples of normal psoriatic involved and psoriatic uninvolved skin. There was also no significant difference in the cyclophilin content and cyclophilin mRNA in cultured, normal and psoriatic keratinocytes, thus confirming that keratinocytes contain cyclophilin and that the epidermal values for cyclophilin content are not skewed by the intra-epidermal leucocyte content. The similarity of cyclophilin mRNA levels in epidermis and cultured KCs (data not shown) make it a suitable reference gene for steady-state mRNA analysis.

The presence of cyclophilin within the keratinocyte would explain the high epidermal accumulation of CsA, 10 times serum trough levels, reported in psoriatic patients treated with oral CsA [15]. The therapeutic effect of CsA on psoriatic skin appears to be independent of the cyclophilin content. In psoriasis, CsA most probably exerts its therapeutic effect via immunosuppression, namely, by inhibition of CD4 T-lymphocyte proliferation and function, as has been described in the skin of psoriatic patients treated with low-dose oral CsA [22].

12-O-tetradecanoyl-phorbol-13-acetate (TPA) induces epidermal hyperproliferation in the hairless mouse [23] and this has been likened to the hyperproliferative process observed in psoriasis [24]. Gschwendt et al [25,26], using high doses of CsA, and Gupta et al [27] have demonstrated that CsA inhibits this TPA-induced process and have suggested that this ability may be separable from its immunosuppressive properties. It has been postulated that the inhibition of TPA effects by CsA may involve suppression of calmodulin-dependent processes such as phosphorylation of elongation factor-2, which may be necessary for TPA-induced protein synthesis in vivo. On this basis CsA could be working in psoriasis via a dual pathway: immunosuppression and directly anti-proliferative. The recent reports [10,11] that cyclophilin is identical with PPIase, an enzyme important in the catalysis of protein folding, opens up new avenues for cyclophilin research. Indeed, it has already been proposed [10] that CsA may mediate some of its effects, such as inhibition of interleukin-2 production, by an inhibitory action on PPIase.

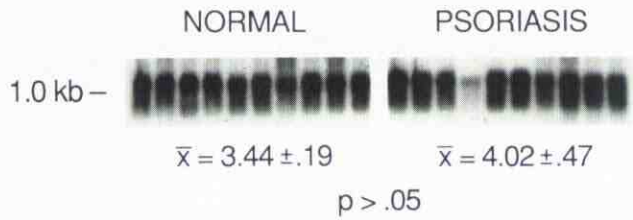

Figure 6. RNA blot analysis of cyclophilin mRNA. Electrophoresis was on a 1.0% formaldehyde agarose gel. Results of a 2-h exposure using 0.5×10^6 cpm/ml hybridization solution are shown.

The finding of cyclophilin in keratinocytes does not imply that CsA is capable of producing a direct effect on these cells, only that CsA may be directly bound to the keratinocyte. The propensity of CsA to directly inhibit keratinocyte proliferation is debatable. Recently Furue et al [28] have shown that CsA inhibits proliferation of transformed and normal murine keratinocytes. Previous workers have not been able to demonstrate this phenomenon [12,29] other than in keratinocytes grown in serum-free medium [30]. Whether cyclophilin or calmodulin participate in this direct anti-proliferative effect of CsA in cultured keratinocytes remains to be determined.

In conclusion, cyclophilin is present in normal and psoriatic epidermis in equivalent amounts and is probably responsible, at least in part, for the epidermal binding of CsA. The exact roles of cyclophilin in epidermal physiology and in inhibition of keratinocyte proliferation by CsA remain to be elucidated, particularly with regard to the therapeutic mechanism of action of CsA in psoriasis.

REFERENCES

1. Borel JF, Feurer C, Gubler HU, Stahlein H: Biological effects of cyclosporin A: a new antilymphocytic agent. Agents Actions 6:468–475, 1976
2. Griffiths CEM, Powles AV, Leonard JN, Fry L, Baker BS, Valdimarsson H: Clearance of psoriasis with low dose cyclosporin. Br Med J 293:731–732, 1986
3. Ellis CN, Gorsulowsky DC, Hamilton TA, Billings JK, Brown MD, Headington JT, Cooper KD, Baadsgaard O, Duell EA, Annesley TM, Turcotte JG, Voorhees JJ: Cyclosporine improves psoriasis in a double-blind study. JAMA 256:3110–3116, 1986
4. Bunjes D, Hardt C, Rollinghof M, Wagner H: Cyclosporin A mediates immunosuppression of primary cytotoxic T cell responses by impairing the release of interleukin-1 and interleukin-2. Eur J Immunol 11:657–661, 1981
5. Elliot JF, Lin Y, Mizel SB, Bleackley RC, Harnish DG, Paetkau V: Induction of interleukin 2 messenger RNA inhibited by cyclosporin A. Science 226:1439–1441, 1984
6. Kalman VK, Klimpel GR: Cyclosporin A inhibits the production of gamma interferon (IFN-γ) but does not inhibit production of virus-induced IFN. Cell Immunol 78:122–129, 1983
7. Harding MW, Handschumacher RE, Speicher DW: Isolation and amino acid sequence of cyclophilin. J Bio Chem 261:8547–8555, 1986
8. Danielson PE, Forss-Petter S, Brow MA, Calvetta L, Douglass J, Milner RJ, Sutcliffe JG: p-1B15: A cDNA clone of the rat mRNA encoding cyclophilin. DNA 7:261–267, 1988
9. Koletsky AJ, Harding MW, Handschumacher RE: Cyclophilin: distribution and variant properties in normal and neoplastic tissues. J Immunol 137:1054–1059, 1986
10. Takahashi N, Hoyano T, Suzuki M: Peptidyl-prolyl cis-trans isomerase is the cyclosporin A-binding protein cyclophilin. Nature 337:473–475, 1989
11. Fischer G, Wittman-Liebold B, Lang K, Kiefhaber T, Schmid FX: Cyclophilin and peptidyl-prolyl cis-trans isomerase are probably identical proteins. Nature 337:476–478, 1989
12. Colombani PN, Robb A, Hess AD: Cyclosporin A binding to calmodulin: a possible site of action on T lymphocytes. Science 228:337–339, 1985
13. Foxwell BMJ, Hiestand PC, Wenger R, Ryffel B: A comparison of cyclophilin and calmodulin and the identification of a novel 45 Kd cyclosporine-binding phosphoprotein in Jurkat cells. Transplantation 46:35s–40s, 1988

14. Russell DH, Matrisian L, Kibler R, Larson DF, Poulos B, Magun BE: Prolactin receptors on human lymphocytes and their modulation by cyclosporine. Biochem Biophys Res Commun 121:899–906, 1984
15. Fisher GJ, Duell EA, Nickoloff BJ, Annesley TM, Kowalke JK, Ellis CN, Voorhees JJ: Levels of cyclosporine in epidermis of treated psoriasis patients differentially inhibit growth of keratinocytes cultured in serum free versus serum-containing media. J Invest Dermatol 91:142–146, 1988
16. Merker MM, Handschumacher RE: Uptake and nature of the intracellular binding of cyclosporin A in a murine thynoma cell line BW5147. J Immunol 132:3064–3070, 1984
17. Handschumacher RE, Harding MW, Rice J, Drugge RJ, Speicher DW: Cyclophilin: A specific cytosolic binding protein for cyclosporin A. Science 226:544–547, 1984
18. Bradford M: A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 72:248–254, 1976
19. Boyce ST, Ham RG: Calcium regulated differentiation of normal human epidermal keratinocytes in chemically defined clonal culture and serum free serial culture. J Invest Dermatol 81:33S–40S, 1983
20. Towbin H, Staehelin T, Gordon J: Electrophoretic transfer of proteins from polyacrylamide gel to nitrocellulose sheets: Procedure and some applications. Proc Natl Acad Sci USA 76:4350–4354, 1979
21. Elder JT, Fisher GJ, Lindquist PB, Bennett GL, Pittelkow MR, Coffey RJ, Ellingsworth L, Derynck R, Voorhees JJ: Overexpression of transforming growth factor-α in psoriatic epidermis. Science 243:811–814, 1989
22. Baker BS, Griffiths CEM, Lambert S, Powles AV, Valdimarsson H, Fry L: Cyclosporin: It's effects on T and dendritic cells in chronic plaque psoriasis. Br J Dermatol 116:503–510, 1987
23. Marks F: Prostaglandins, cyclic nucleotides and the effect of phorbol ester tumor promoters on mouse skin in vivo. Carcinogenesis 4:1465–1470, 1983
24. Horn F, Marks F, Fisher GJ, Marcelo CL, Voorhees JJ: Decreased protein kinase C activity in psoriatic versus normal epidermis. J Invest Dermatol 88:220–222, 1987
25. Gschwendt M, Kittstein W, Marks F: The weak immunosuppressant cyclosporine D as well as the immunologically inactive cyclosporine H are potent inhibitors in vivo of phorbol ester TPA induced biological effects in mouse skin and of Ca^{2+}/calmodulin dependent EF-2 phosphorylation in vitro. Biochem Biophys Res Commun 150:545–551, 1988
26. Gschwendt M, Kittstein W, Marks W: Cyclosporin A inhibits phorbol ester-induced cellular proliferation and tumor promotion as well as phosphorylation of a 100-Dd protein in mouse epidermis. Carcinogenesis 8:203–207, 1984
27. Gupta AK, Fisher GJ, Elder JT, Talwar HS, Esmann J, Duell EA, Nickoloff BJ, Voorhees JJ: Topical cyclosporine A inhibits the phorbol ester induced hyperplastic inflammatory response but not protein kinase C activation in mouse epidermis. J Invest Dermatol 93:379–386, 1989
28. Furue M, Gaspari AA, Katz SI: The effect of cyclosporin A on epidermal cells. II. Cyclosporin A inhibits proliferation of normal and transformed keratinocytes. J Invest Dermatol 90:796–800, 1988
29. Kato N, Halprin KM, Taylor JR: Cyclosporin A does not inhibit epidermal cell growth at therapeutic levels. J Invest Dermatol 88:52–54, 1987
30. Nickoloff BJ, Fisher GJ, Mitra R, Voorhees JJ: Additive and synergistic antiproliferative effects of cyclosporin A and gamma interferon on cultured human keratinocytes. Am J Pathol 131:12–18, 1988

ANNOUNCEMENT

The 9th CIRD Symposium, "Advances in Skin Pharmacology: Immunological and Pharmacological Aspects of Atopic and Contact Eczema," will be held October 4–6, 1990 in Cannes, France. Send abstracts of approximately 300 words for formal or poster presentations to: Dr. J. Czernielewski, CIRD, Sophia Antipolis, F-06565 Valbonne, France.